Bacteriocins and Bacteriophage Lytic Proteins as Alternatives to Antibiotics from Russian Federation and USA Collaborations

Session 1

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ABSTRACT

Novel anti-microbial peptides (bacteriocins) were isolated and characterized during collaborative research between PMSRU, ARS-USDA scientists and representatives of the State Research Center for Applied Microbiology and Biotechnology (SRCAMB) in Obolensk, Russian Federation. The bacteriocins are effective against several bacterial pathogens. Treatment of chickens by feeding bacteriocins consistently reduced *Campylobacter* levels in their gastrointestinal system as compared with levels found in untreated birds. Five patents have been issued describing this alternative to antibiotics treatment for bacterial infection and technology transfer is on-going. Screening of bacteriophages lytic for *Clostridium perfringens* was completed utilizing filtered samples obtained from poultry (intestinal material), soil, sewage and poultry processing drainage water. From the collections highly lytic viruses were isolated and the double-stranded deoxyribonucleic acid (DNA) genomes of the bacteriophages were sequenced to completion. DNA sequencing of six bacteriophage genomes completed at PMSRU and four genomes in collaboration with Russian investigators resulted in identification of unique amidases as well as phage encoded proteins that potentially contain lysozyme and endopeptidase activities. Two recombinant bacteriophage lytic enzyme genes encoding putative amidases have been cloned, their proteins expressed as recombinants and isolated to homogeneity, then demonstrated to lyse *C. perfringens*. Patent applications have been submitted as a result of the bacteriophage research. These bacteriocins and phage lytic enzymes may have possibilities for use in agriculture and medical applications as potential replacements for current antibiotics that may have diminished activity.

INTRODUCTION

The World Health Organization (WHO) reports that governments worldwide are intensifying their efforts (http://www.who.int/mediacentre/factsheets/fs237/en/) to improve food safety. Internationally food-borne disease is difficult to estimate, but it has been reported that in 2005 alone 1.8 million people died from diarrheal diseases and a great proportion of these cases were attributed to contamination of food and drinking water (WHO, 2010 web site). The majority of pathogens causing this significant disease burden are considered to be zoonotic (Schlundt et al., 2004). The most important factors driving an increase in the burden of food-borne disease during the future are anticipated to be a doubling of the global demand for food accompanied by increased international trade in food. This will be accompanied by an increase in consumption of certain high-value food commodities such as meat, poultry and fresh produce. One of the most important factors in reducing the burden of food-borne disease was identified as development of effective control measures (Quested et al., 2010). The USA Centers for Disease Control and Prevention estimates that in the United States alone *Campylobacter* spp., *Salmonella* spp. (non-typhoid), and CPE-producing Type A *Clostridium perfringens*, are the three leading bacterial etiologies of human food-borne illness with 1.32 million, 1.23 million and 0.97 million domestic cases respectively (Scallan et al., 2011). The application of therapeutic bacteriocin treatments to reduce poultry colonization diminishes *Campylobacter* spp. in cecal material to low levels in treated birds and this could be a valid means for controlling food-borne bacteria in poultry (Svetoch and Stern, 2010). Also, bacteriophage gene products may be another avenue for developing alternative antimicrobials to control pathogenic bacteria (Liu et al., 2004).

METHODS

Bacteriocins were purified from lactic acid bacteria isolated from chicken gastrointestinal materials. The bacteriocins were then assayed for their antimicrobial properties (Line et al., 2008; Stern et al., 2006; Svetoch et al., 2011). Bacteriophages that infected *C. perfringens* were isolated from poultry intestinal material, poultry processing offal and sewage (Oakley et al., 2011; Volozhantsev et al., 2011; Volozhantsev et al., 2012). Recombinant lytic proteins were subsequently expressed and assayed for the ability to lyse *C. perfringens* (Simmons et al., 2010).

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RESULTS

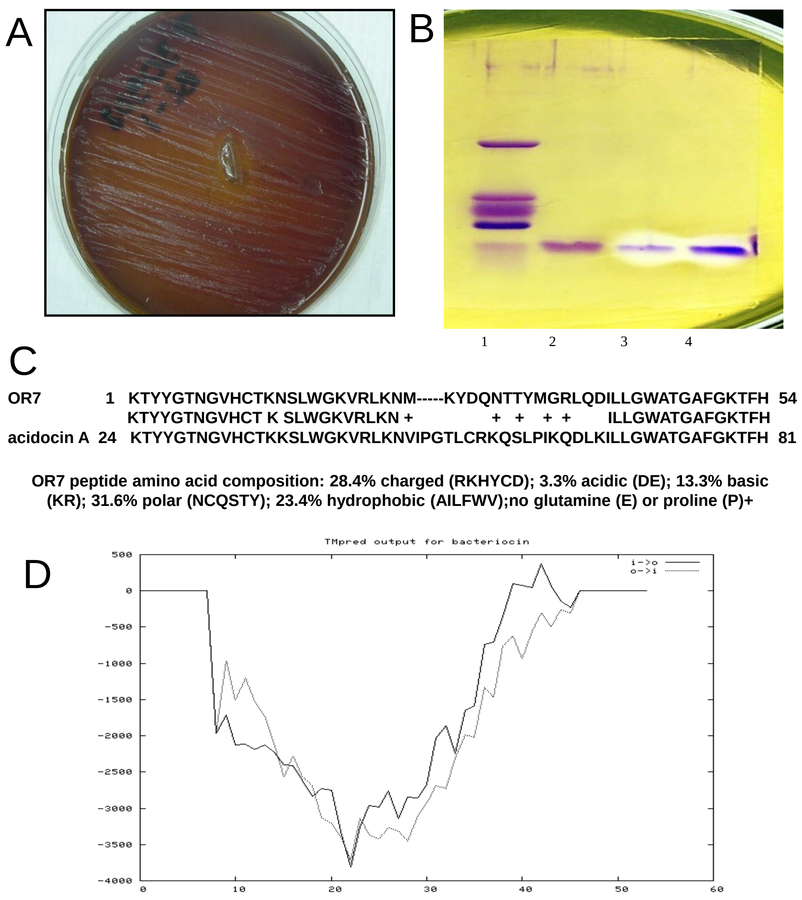

Fig 1. Bacteriocin characteristics (A) Lactic acid bacteria selected for inhibition of *Campylobacter jejuni* growth. (B) SDS-PAGE of purified bacteriocin with bacterial overlay. (C) Alignment and biochemical characteristics of common bacteriocins. (D) Transmembrane domain prediction for bacteriocin OR7.

Table 1. Ten chicks/group were colonized on day of hatch with 10^{10} CFU *Campylobacter jejuni* and treated with OR-7 for 10 days.

Trial and Treatment	Campy challenge strain	Treatment days	Mean $\log_{10}$ CFU/gm cecum
1 Control	AL-22	0	7.2±0.3
1 Treated	AL-22	7-9	ND
2 Control	BH-6	0	7.1±0.4
2 Treated	BH-6	7-9	0.7±1.2
3 Control	BL-1	0	7.8±0.2
3 Treated	BL-1	7-9	1.3±1.8
4 Control	CL-11	0	6.6±0.7
4 Treated	CL-11	7-9	ND

NOTE: During chicken feeding trials, bacteriocin treatment significantly reduced the numbers of *C. jejuni* organisms compared to those found in the untreated control groups of birds ($P \leq 0.05$; ND-not detected).

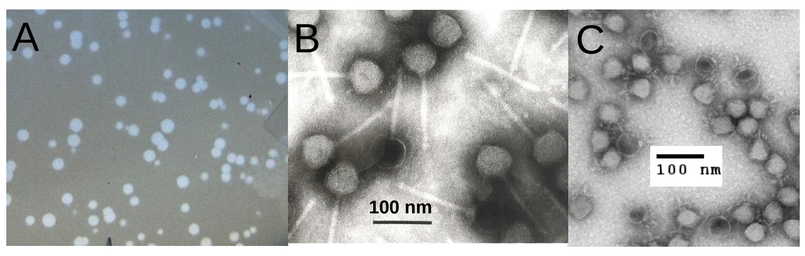

Fig 2. Representative clear plaques and electron micrographs for Russian and USA bacteriophages. (A) All phages were plaque purified at least 3X. (B) Many phages had long non-contractile tails representative of the *Siphoviridae.* (C) Several phages were representative of the *Podoviridae* with short non-contractile tails.

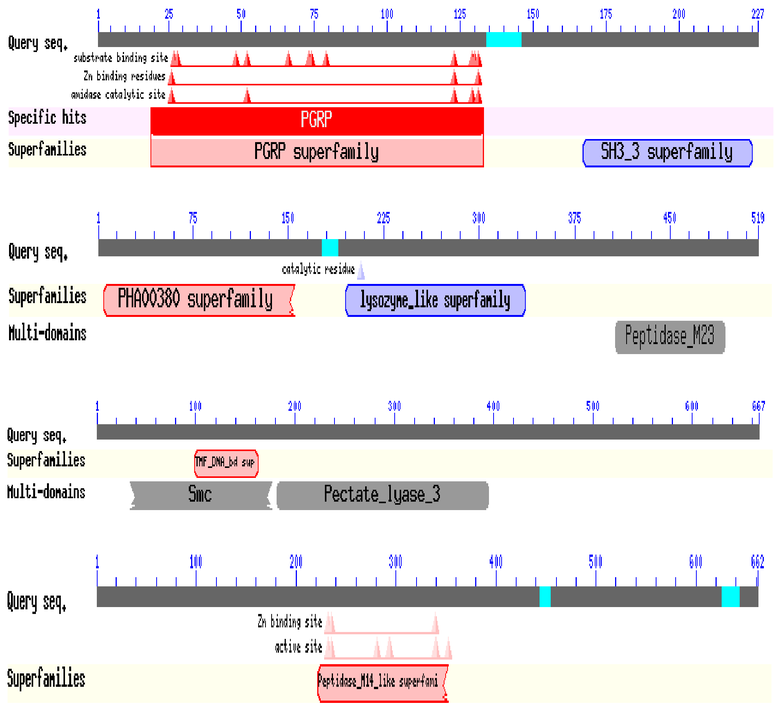

Fig 3. Bacteriophage proteins identified with potential to lyse *Clostridium perfringens*. Includes N-acetylmuramoyl-L-alanine amidases with C-terminal cell wall binding domains, tail proteins with lysozyme and peptidase activities as well as a pectate lyase and a previously unknown viral Zn-endopeptidase.

RESULTS CONTINUED

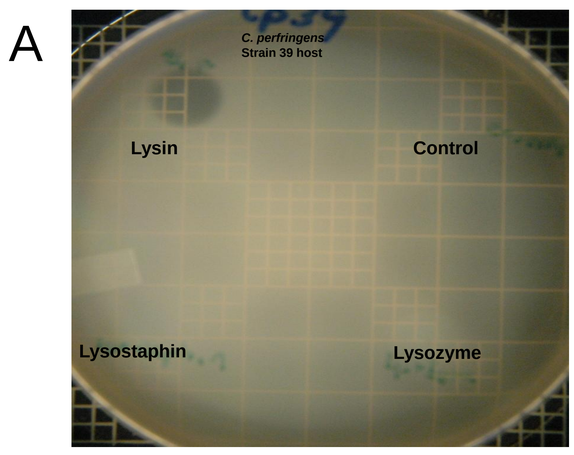

Fig 4. Spot lysis assay of PlyCP39O/CP26Fand Gram stain of *C. perfringens* lysin-treated cells. (A) Spot assay and (B) cells treated without phage lysin or (C) with lysin.

CONCLUSIONS

- Bacteriocins were discovered with activity against the food-borne pathogen *Campylobacter jejuni*.
- Bacteriophages encoded proteins as potential antimicrobials to lyse *Clostridium perfringens*.
- Future need is expression in yeast with large-scale production for field trials in food animals.

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